10/530849
JC12 Rec'd PCT/PTC 08 APR 2005

Pharmaceutically active oligosaccharide conjugates

The present invention relates to pharmaceutically active oligosaccharide conjugates having the formula $(X–Y_m)_n$- S, wherein component X is a pharmaceutically active compound, Y is a bifunctional linker, and S is an oligosaccharide, consisting of 1 to 20 saccharide units, n is equal or less than the number of the saccharide units in the oligosaccharide S, and m is, independent of n, 0 or 1.
In addition, the present invention is directed to a process of preparing compounds of the present invention, comprising the step of coupling components X and S directly or indirectly by means of a bifunctional linker group.
Furthermore, the present invention relates to the use of said pharmaceutically active oligosaccharide conjugates as a medicament as well as to pharmaceutical compositions, freeze-dried pharmaceutical compositions, and a kit, all of which comprise at least one of said pharmaceutically active oligosaccharide conjugates.

Field of the invention

Many low molecular weight pharmaceutically active molecules are drastically limited in their medical application or even not used at all because of an insufficient solubility in aqueous solutions. Besides other undesirable effects this often results in a substantial decrease in bioavailability. In spite of this drawback, some drugs are administered because of a lack of suitable alternatives. In those instances the galenic formulation may, e.g., be an oily bolus or an emulsion, both of which often result in a painful deposition thereof at the injection site.

As an even more severe side effect, a lack in solubility can lead to accumulation phenomena of the drug in one or more body compartments or organs (liver, kidney, etc.) which is generally accompanied by toxic side effects. In addition, low solubility frequently implicates a very narrow therapeutic range, resulting in a low value of the therapeutic index.

There have been a number of more or less successful strategies to overcome the drawbacks of insolubility such as, e.g., drug entrapment in soluble or insoluble matrices, liposomes targeted administration, and nanoparticles. An elegant way to solve this problem is by coupling the insoluble medical substance to a large biocompatible

hydrophilic polymer, such as polyethylene glycol (PEG), dextran, starch or other watersoluble polymers.

Presently, the most widely used polymer in this respect is PEG because dextran conjugates often elicit allergic reactions in clinical application. However, PEG-conjugates have been observed to sometimes lead to side effects such as, e.g., itching, hypersensibility reactions and pancreatitis.

Hydroxyethyl starch (HES) has a more promising biocompatibility profile, and has a well-known, predictable pharmacokinetic behaviour. Moreover, it is much more versatile in terms of its molecular weight availability (e.g. it may be produced from 10 kD to > 500 kD) than synthetic polymers like PEG. It has also been accepted as being safer than PEG due to its well investigated degradation pathway.

Nevertheless, hydroxyethyl starch shares a common disadvantage with all other presently available polymers: its polydispersity. The polymer conjugates are always a mixture of molecules having molecular weights distributed around an average value. This lack of homogeneity results in a low level of chemical and biochemical characterization. Moreover, the polymer component may prevent the pharmaceutically active component to reach its site of action (receptor, enzyme, etc.). In these cases the drug to be active requires its delivery in the original unconjugated form, and thus cleavage of the polymer by metabolic reactions is required for its pharmaceutical efficacy.

In summary, there is still a need for stable and water soluble derivatives of pharmaceutically active compounds having an improved pharmacokinetic profile and biocompatibility in comparison to the pharmaceutically active components of the conjugates alone. Improved conjugates can be capable of hydrolytic activation under physiological conditions. Specifically, there is a need for stable and water soluble conjugates as prodrugs that can be readily metabolized to release the pharmaceutically active component *in vivo*. In addition, there is a need for stable and water soluble derivatives of pharmaceutically active compounds having an improved pharmacokinetic profil that are pharmaceutically active as conjugates and/or released slowly from the conjugate as to provide a delayed release form and a steric protection from metabolic enzymes.

In one aspect the present invention provides a compound having the formula:

$$(X - Y_m)_n - S,$$

wherein

X is a pharmaceutically active compound,

Y is a bifunctional linker,

S is an oligosaccharide, consisting of 1 to 20 saccharide units,

n is equal or less than the number of the saccharide units in the oligosaccharide S, and

m is, independently of n, 0 or 1.

Preferably, n is 1 to 8, more preferably 1 to 3, and most preferably 1.

In a preferred embodiment m is 0 and X and S are linked to each other by an amide, imine, secondary or tertiary amine, ether, ester, carbonate, carbamate, urea or thioester bond.

More preferably, components X and S are linked to each other by a bond

(i) involving an oxygen, nitrogen, or sulfur of component X and a carbon derivative of component S, or

(ii) involving a oxygen, nitrogen, or sulfur of a saccharide of component S and a carbon derivative of component X.

The term carbon derivative as used herein relates to those carbon derivatives that are comprised in an amide, imine, secondary or tertiary amine, ether, ester, carbonate, carbamate, urea or thioester bond.

In an alternative preferred embodiment, the present invention relates to compounds of the invention, wherein m is 1 and X and S are linked by means of a pharmaceutically

acceptable linking group Y, said linking group Y preferably being linked to X and S by an amide, imine, secondary or teriary amine, ether, ester, carbonate, carbamate, urea or thioester bond and wherein the X-Y bond may be different from the Y-S bond.

The term "oligosaccharide" as used herein is defined as encompassing 1 to 20 saccharides. It is emphasized that mono-, di-, and trisaccharides are specifically included in the definition of oligosaccharides.

It was surprisingly found that many of the known insoluble drugs do not require large hydrophilic polymers to produce the desired hydrophilicity in a drug conjugate. Unexpectedly, 1 to 20 saccharide units are found to be sufficient. Conjugates according to the present invention can easily be produced with the homogeneity that is necessary for a predictable and desirable pharmacokinetic profile as well as enhanced biocompatibility.

In a preferred embodiment, S consists of 1 to 10, preferably of 2 to 7 saccharide units.

The oligosaccharide S may be linear or branched and the saccharide units within the oligosaccharide are linked to each other by α- or ß(1-2), (1-4), or (1-6) bonds.

Preferably the oligosaccharide is linear, and more preferably the oligosaccharide is linear and the saccharide units within the oligosaccharide are linked by α- or ß(1-4) bonds. In the most prefered embodiment, the oligosaccharide is linear and the saccharide units within the oligosaccharide are linked by α(1-4) bonds.

According to the invention it is preferred that one or more pharmaceutical component(s) X is (are) linked to a terminal saccharide unit(s) of the oligosaccharide S.

The term "terminal saccharide unit" as used herein refers to a saccharide unit that is linked to none or only one further saccharide unit in S.

In a preferred embodiment, the oligosaccharide S comprises aldose saccharide units, preferably terminal aldose saccharide units having a free reducing end. More preferably, oligosaccharide S comprises at least one saccharide unit that is linked to a compound X that is derived from an aldose monosaccharide comprising a free aldehyde group.

When using smaller oligosaccharides according to this invention yet another important advantage is the possibility to solubilize a much higher amount of the pharmaceutically active substance without yielding highly viscous solutions, that are generally observed for polymer-conjugated small molecules at high concentrations. For example, a trisaccharide (e.g., maltotrionic acid) conjugated drug in solution will achieve an almost 100 times higher concentration compared to the same drug coupled to hydroxyethyl starch with 50 kD molar mass before reaching an unacceptable viscosity value. Therefore, higher concentrations of the therapeutic component can be reached much easier with the conjugates according to this invention. As a consequence, conjugates of the invention are not only easier to handle for galenic formulations (e.g. reduced side effects such as, e.g., reduced deposition of the conjugate at the site of administration and reduced accumulation in undesired locations in a body) and clinical applications but also allow a higher therapeutic dosage in comparison to HES or PEG conjugates.

In a preferred embodiment the viscosity of conjugates according to the invention is 1-100 mPasc (Pascal x s), preferably 1-10 mPasc, more preferably 1-7 mPasc. For a review on viscosity in relation to physiology see J.D. Bronzine The biomedical engineering handbook, CRC Press, USA, Salem, 1995.

In a preferred embodiment the molar ratio of X to S is in the range of 20:1 to 1:1, preferably in the range of 15:1 to 1:1, more preferably in the range of 5:1 to 1:1. Most preferably the ratio of X to S is 1:1.

Also preferred are conjugates comprising structurally different pharmaceutically active components X. Preferably, the conjugates comprise 1 to 3 structurally different components x, more preferably 1 component X.

In a preferred embodiment, the oligosaccharide S comprises one or more of the oligosaccharide unit(s) which is (are) identical or different and each selected from the group consisting of:

a) monosaccharides, preferably: ribose, arabinose, xylose, lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, talose, fucose;
b) disaccharides, preferably lactose, maltose, isomaltose, cellobiose, gentiobiose, , melibiose, primeverose, rutinose;

c) disaccharide homologues, preferably maltotriose, isomaltotriose, maltotetraose, isomaltotetraose, maltopentaose, maltohexaose, maltoheptaose, lactotriose, lactotetraose;
d) uronic acids, preferably glucuronic acid, galacturonic acid;
e) branched oligosaccharides, preferably panose, isopanose,
f) amino monosaccharides, preferably galactosamine, glucosamine, mannosamine, fucosamine, quinovosamine, neuraminic acid, muramic acid;, lactosediamine, acosamine, bacillosamine, daunosamine, desosamine, forosamine, garosamine, kanosamine, kansosamine, mycaminose, mycosamine, perosamine, pneumosamine, purpurosamine, rhodosamine;
g) modified saccharides, preferably abequose, amicetose, arcanose, ascarylose, boivinose, chacotriose, chalcose, cladinose, colitose, cymarose,2-deoxyribose, 2-deoxyglucose, diginose, digitalose, digitoxose, evalose, evernitrose, hamamelose, manninotriose, melibiose, mycarose, mycinose, nigerose, noviose, oleandrose, paratose, rhodinose, rutinose,sarmentose, sedoheptulose, solatriose, sophorose, streptose, turanose, tyvelose.

In a more preferred embodiment, S comprises one or more of the saccharide unit(s) which is (are) selected from the group consisting of glucose, galactose, glucosamine, galactosamine, glucuronic acid, gluconic acid, galacturonic acid, lactose, lactotetraose, maltose, maltotriose, maltotetraose, isomaltose, isomaltotriose, isomaltotetraose, and neuraminic acid.

The pharmaceutically active compound X may be any drug compound or vitamin that lacks a desirable water solubility.

Preferably, the pharmaceutically active compound X is selected from the group consisting of:
antibiotic, anti-diabetic, anti-diuretic, anti-cholinergic, anti-arrhytmic, anti-emetic, anti-epileptic, anti-histaminic, anti-mycotic, anti-sympathotonic, anti-thrombotic, androgenic, anti-androgenic, estrogenic, anti-estrogenic, anti-osteoporotic, anti-cancer, immuno-suppressing, vasodilatory antipyretic, analgesic, anti-inflammatory drugs, blood pressure lowering drugs, antitussiva, antidepressiva, ß-blockers, and vitamins.

More preferably, the pharmaceutically active compound X is selected from the group consisting of:

a) drugs comprising a primary amino group, preferably selected from the group consisting of :
Albuterol, Alendronat, Amikazin, Ampicillin, Amoxicillin, Amphotericin B, Atenolol, Azathioprin, Cefaclor, Cefadroxil, Cefotaxim, Ceftazidim, Ceftriaxon, Cilastatin, Cimetidin, Ciprofloxacin, Clonidin, Colistin, Cosyntropin, Cycloserin, Daunorubicin, Doxorubicin, Desmopressin, Dihydroergotamin, Dobutamin, Dopamin, Ephedrin, Epinephrin, ε-Aminocapronsäure, Ergometrin, Esmolol, Famotidin, Flecainid, Folsäure, Flucytosin, Furosemid, Ganciclovir, Gentamicin, Glucagon, Hydrazalin, Imipenem, Isoproterenol, Ketamin, Liothyronin, Merpatricin, Metaraminol, Methyldopa, Metoclopramid, Metoprolol, Mexiletin, Mitomycin, Neomicin, Netilmicin, Nimodipin, Nystatin, Octreotid, Oxytocin, Pamidronat, Pentamidin, Phentolamin, Phenylephrin, Procainamid, Procain, Propranolol, Ritodrin, Sotalol, Teicoplanin, Terbutalin, Thiamin, Tiludronat, Tolazolin, Trimethoprim, Tromethamin, Vancomycin, Vasopressin, and Vinblastin;

b) drugs comprising a carboxylic acid group, preferably selected from the group consisting of:
Acetylcystein, Azlocillin, Aztreonam, Benzylpenicillin, Camptothecin, Cefamandol, Cefazolin, Cefepim, Cefotaxim, Cefotetan, Cefoxitin, Ceftazidim, Ceftriaxon, Cephalothin, Cilastatin, Ciprofloxacin, Clavulansäure, Dicloxacillin, ε-Amino-capronsäure, Floxacillin, Folinsäure, Furosemid, Fusidinsäure, Imipemem, Indomethacin, Ketorolac, Liothyronin, Melphalan, Methyldopa, Piperacillin, Prostacyclin, Prostaglandine, Teicoplanin, Ticarcillin and Vancomycin.

c) drugs comprising an arylic –OH group, preferably selected from the group consisting of:
Albuterol, Allopurinol, Apomorphin, Ceftriaxon, Dobutamin, Dopamin, Doxycyclin, Edrophonium, Isoproterenol, Liothyronin, Metaraminol, Methyldopa, Minocyclin, Pentazocin, Phenylephrin, Phentolamin, Propofol, Rifamycine, Ritodrin, Teicoplanin, Terbutalin, Tetracyclin and Vancomycin.

d) drugs comprising an aliphatic –OH group, preferably selected from the group consisting of Cyclosporin, Taxol and Paclitaxel.

In a preferred embodiment, the compounds of the present invention comprise a bifunctional linker, wherein the bifunctional linker selected from the group consisting of:

a) linker molecules that connect an –SH group with an amino group, preferably derived from a compound selected from the group consisting of:

AMAS	(N-α(Maleimidoacetoxy)succinimide ester),
BMPS	(N-ß(Maleimidopropyloxy)succinimide ester),
GMBS	(N-γ(Maleimidobutyryloxy)succinimide ester),
EMCS	(N-ε(Maleimidocaproyloxy)succinimide ester),
MBS	(m-Maleimidobenzoyl-N-hydroxysuccinimide ester),
SMCC	(Succinimidyl4-(N-maleimidomethyl)-cyclohexane-1-carboxylate),
SMPB	(Succinimidyl-4-(p-maleimidophenyl) butyrate),
SPDP	(Succinimidyl-3-(2-pyridyldithio) propionate),
Sulfo-GMBS	(N-(γ-Maleimidobutyryloxy)sulfosuccinimide ester), and
Sulfo-EMCS	(N-(ε-Maleimidocaproyloxy)sulfosuccinimide ester);

b) linker molecules that connect two –SH groups, preferably derived from a compound selected from the group consisting of:

BMB	(1.4-Bis-maleimidobutane),
BMDB	(1.4-Bis-maleimido-2.3-dihydroxybutane),
BMH	(Bis-maleimidohexane),
BMOE	(Bis-maleimidoethane),
DTME	(Dithio-bis-maleimidoethane),
HBVS	(1.6-Hexane-bis-vinylsulfone),
$BM(PEO)_3$	(1.8-Bis-maleimidotriethyleneglycol), and
$BM(PEO)_4$	(1.11-Bis-maleimidotetraethyleneglycol);

c) linker molecules that connect two amino groups, preferably derived from a compound selected from the group consisting of:

BSOCOES	(Bis-(2-(succinimidyloxycarbonyloxy)-ethyl)sulfone,

BS^3	(Bis-(sulfosuccinimidyl)suberateDFDNB (1.5-Difluoro-2.4-dinitrobenzene),
DMA	(Dimethyladipimidate 2 HCl),
DSG	(Disuccinimidyl glutarate),
DSS	(Disuccinimidyl suberate), and
EGS	(Ethylene glycol bis(succinimidylsuccinate),

d) linker molecules that connect an –SH group and a –CHO functional groug, preferably derived from a compound selected from the group consisting of:

BMPH	(N-(ß-Maleimidopropionic acid)hydrazide TFA),
EMCH	(N-(ε-Maleimidocaproic acid)hydrazide),
KMUH	(N-(κ-Maleimidoundecanoic acid)hydrazide),
M_2C_2H	(4-(N-Maleimidomethyl)cyclohexane-1-carboxylhydrazide HCl),
MPBH	(4-(4-N-Maleimidophenyl)butyric acid hydrazide HCl), and
PDPH	(3-(2-Pyridyldithio)propionyl hydrazide),

e) linker molecules that connect an –SH group to an –OH group, preferably a compound derived from PMPI (N-(p-Maleimidophenyl)isocyanate);

f) linker molecules that connect an –SH group to a –COOH group, preferably derived from a compound selected from the group consisting of:

BMPA	(N-ß-Maleimidopropionic acid),
EMCA	(N-ε-Maleimidocaproic acid), and
KMUA	(N-κ-Maleimidoundecanoic acid);

g) linker molecules that transform an amino group into a carboxyl group, preferably derived from a compound selected from the group consisting of: MSA (Methyl-N-succinimidyladipate) and its longer and shorter chain homologues or the corresponding ethylene glycol derivatives;

h) linker molecules that transform a –COOH group into an amino group, preferably derived from a compound selected from the group consisting of: DAB (1.4-Diaminobutane) or its longer and shorter chain homologues or the corresponding ethylene glycol derivatives.

For the skilled person the preparation of the conjugates of the present invention is within his average skill and merely requires routine experimentation and optimization of standard synthesis strategies that are abundantly available in the prior art. Numerous non-degrading and selective strategies are available for linking amine, alcohol, and thiol functional groups with aldehyde, carboxylic acid or activated carboxylic acid functional groups. If component X and/or S lack the desired functional group it may be introduced by chemical derivatization of existing functional groups, the addition of suitable functional groups, or the addition of suitable functional linker molecules.

In a further aspect, the present invention relates to a process for preparing compounds according to the present invention, comprising the steps of:

a) coupling one or more pharmaceutically active compound(s) X, comprising an amino, alcohol, and/or thiol group, with one or more aldehyde group(s) of an oligosaccharide S, or

b) coupling one or more pharmaceutically active compound(s) X, comprising an amino, alcohol, and/or thiol group with one or more carboxylic group(s) of an oligosaccharide S, or

c) coupling one or more pharmaceutically active compound(s) X, comprising an amino, alcohol, and/or thiol group with one or more activated carboxylic group(s) of an oligosaccharide S, or

d) coupling one or more pharmaceutically active compound(s) X comprising a carboxyl and/or aldehyde functional group with one or more amino, thiol, or alcohol group(s) of an oligosaccharide S.

The carboxyl group can be used as such or after a previous activation step, that yields an activated carboxylic acid group, such as, e.g. a lactone, an active ester, a symmetric

anhydride, a mixed anhydride, a halogenide of a carboxylic acid or any other activated form of a carboxylic group that is suitable to produce the desired ester bond.

Preferred examples of activated carboxylic acids that may be used to practice specific embodiments of the present invention are selected from the group consisting of a lactone, an anhydride, a mixed anhydride, and a halogenide of a carboxylic acid.

Preferred examples of activated carboxylic acids are selected from the group consisting of a lactone, an anhydride, a mixed anhydride, and a halogenide of a carboxylic acid.

More preferred activated carboxylic acids are esters of p-nitrophenol; 2,4,6-trinitrophenol; p-chlorophenol; 2,4,6-trichlorophenol; pentachlorophenol; p-fluorophenol; 2,4,6-trifluorophenol; pentafluorophenol; N-hydroxybenzotriazole; N-hydroxysuccinimide;

Activated carboxylic acids can, for example, be formed by using one of the following reagents:
N-hydroxy succinimide, N-hydroxy phthalimide, thiophenol, p-nitrophenol, o,p-dinitrophenol, trichlorophenol, trifluorophenol, pentachlorophenol, pentafluorophenol, 1-hydroxy-1H-benzotriazole (HOBt), HOOBt, HNSA, 2-hydroxy pyridine, 3-hydroxy pyridine, 3,4-dihydro-4-oxobenzotriazin-3-ol, 4-hydroxy-2,5-diphenyl-3(2H)-thiophenone-1,1-dioxide, 3-phenyl-1-(p-nitrophenyl)-2-pyrazolin-5-one), [1-benzotriazolyl-N-oxy-tris(dimethylamino)-phosphoniumhexa-fluorophosphate] (BOP), [1-benzotriazolyloxytri-pyrrolidinophosphonium-hexafluoro-phosphate (PyBOP), [O-(benzotriazol-1-yl)-N,N,N`,N`-tetramethyluroniumhexa-fluorophosphate (HBTU), [O-(benzotriazol-1-yl)-N,N,N`,N`-tetramethyluronium-tetrafluoroborate (TBTU), [O-(benzotriazol-1-yl)-N,N,N`,N`-bis(pentamethylen)uronium-hexafluorophosphate, [O-(benzotriazol-1-yl)-N,N,N`,N`-bis(tetramethylen)uronium-hexafluorophosphate, carbonyldiimidazole (CDI), carbodiimides, examples are 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC), dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIPC).

When the process of the invention results in the formation of an imine, it is preferred that the process further comprises the step of reducing the imine to a secondary amine.

This is preferably achieved in a single step selective reduction, more preferably by a selective reducing agent selected from the group consisting of:

sodium cyano boro hydride, sodium boro hydride, 4-(dimethylamino)pyridin-borocomplex, N-ethyldiisopropyl-amine-borocomplex, N-ethylmorpholine-borocomplex, N-methyl-morpholine-borocomplex, N-phenylmorpholine-borocomplex, lutidin-boro-complex, triethylamine-borocomplex, trimethyl-amine-borocomplex; sodium triacetate-borohydride, sodium triethyl-borohydride, sodium trimethoxyboro-hydride, potassium tri-sec-butylborohydride (K-selectride), sodium-tri-sec-butylborohydride (N-selectride), lithium-tri-sec-butylborohydride (L-selectride), potassium triamylborohydride (KS-selectride) und lithium-triamylborohydride (LS-selectride), most preferably by sodium cyano boro hydride.

It is especially preferred that the imine is reduced by $NaBH_3CN$ at pH values of 6 – 7.

The functional group involved in the coupling reaction of the process of the present invention can be the aldehyde functional group of one or more saccharide units, preferably one or more terminal saccharide units in the oligosaccharide S. This aldehyde functional group can be used as such or be further chemically modified.

In a preferred embodiment the process of the invention further comprises a step b') or c') prior to step b) or c), respectively, wherein one or more terminal aldehyde group(s) of an oligosaccharide S precursor are selectively oxidized to produce the oligosaccharide S to be used in step b) or c).

Preferred oxidation steps for oxidizing one or more terminal aldehyde group(s) of oligosaccharide S to carboxylic or activated carboxylic group(s) are those using

(i) halogen, preferably I_2, Br_2, in alkaline solution, or
(ii) metal ions, preferably Cu^{++} or Ag^{+}, in alkaline solution, or
(iii) electrochemical oxidation.

The resulting carboxylic group can be used in the coupling reaction to yield an ester, thioester or an amide. The carboxyl group can be used as such or after a previous activation step, that yields an activated carboxylic acid group, such as, e.g. a lactone, an active ester, a symmetric anhydride, a mixed anhydride, a halogenide of a carboxylic acid or any other activated form of a carboxylic group that is suitable to produce the desired ester, thioester or amide bond.

Preferred is a process of the invention, wherein in step c) the one or more activated carboxylic group(s) of an oligosaccharide S are activated carboxylic group(s) selected from the group consisting of a lactone, an anhydride, a mixed anhydride, and a halogenide of a carboxylic acid.

Preferably, the process of the invention is one, wherein in step c) the one or more activated carboxylic group(s) of an oligosaccharide S is (are) a lactone group(s).

Preferably such a lactone group results from the oxidation of a terminal aldehyde group of an aldose. More preferably, the oxidation is performed with I_2 in the presence of NaOH, yielding a carboxylic intermediate group that is transformed into a lactone by water elimination.

With the process of the invention such as the one described in an exemplary fashion in example 1 it is possible to reach almost quantitative yields.

The oligosaccharide lactone derivative is sufficiently active to react with a primary amino function. In contrast to the normal conditions that are used for similar coupling reactions, that usually require the presence of activators, e.g., carbodiimides, it was surprisingly found that the reaction also proceeds readily with high chemical yields without an activator. This is a substantial advantage in that additional purification steps that are necessary for separating the activator and its by-products are redundant.

Preferably, the coupling of a lactone oligosaccharide derivative and one or more pharmaceutically active compound(s) X comprising an amino function is performed in the absence of an activator.

Due to the low stability in water of such lactones and due to the low water solubility of the pharmaceutically active component the reaction is preferably performed in presence of a suitable organic solvent.

Preferred organic solvents are polar non-protic ones (DMF, DMSO, N-methylpyrrolidone and the like) or lower alcohols (i.e. C_{1-10}, e.g MeOH, EtOH, PrOH, *i*-PrOH, n-butanol, iso-butanol, tert-butanol, glycol, glycerol). In specific cases it may also be of advantage to perform the reaction in a heterogeneous liquid phase, e.g. a liquid dispersion or suspension.

The functional groups involved in the coupling of X and S can be a nucleophilic group selected from an alcohol, thiol, and amine functional group and an acceptor functional group, selected from an aldehyde, carboxylic acid, and an activated carboxylic acid functional group, preferably a lactone. Any of the functional groups may be naturally present on a component X or S or may be introduced by chemical transformation, e.g. reductive amination of an oligosaccharide by reacting it with, e.g. a diamine (i.e. hydrazine, DAB or homologues thereof) to yield an amino function).

Another way of transforming and linking functional groups is by means of introducing a bifunctional linker that comprises at least two functional groups that are compatible with the selected components X and S.

In a further aspect the present invention relates to a process for preparing compounds according to the invention, comprising the steps of:

a) coupling a suitable bifunctional linker group(s) to compound X, and
b) coupling the product(s) of step a) with one or more aldehyde, carboxylic acid, or activated carboxylic group(s) of an oligosaccharide S, or

a') coupling a suitable bifunctional linker group(s) to one or more aldehyde, carboxylic acid, or activated carboxylic group(s) of a oligosaccharide S, and
b') coupling the product(s) of step a) with one or more compound(s) X.

When an imine bond is formed between the bifunctional linker group and the component X and/or S it may preferably be further reduced to a secondary amine. Preferably this is achieved in a single step selective reduction, more preferably by a selective reducing agent selected from the group consisting of:
sodium cyano boro hydride, sodium boro hydride, 4-(dimethylamino)pyridin-borocomplex, N-ethyldiisopropyl-amine-borocomplex, N-ethylmorpholine-borocomplex, N-methyl-morpholine-borocomplex, N-phenylmorpholine-borocomplex, lutidin-boro-complex, triethylamine-borocomplex, trimethyl-amine-borocomplex; sodium triacetate-borohydride, sodium triethyl-borohydride, sodium trimethoxyboro-hydride, potassium tri-sec-butylborohydride (K-selectride), sodium-tri-sec-butylborohydride (N-selectride), lithium-tri-sec-butylborohydride (L-selectride), potassium triamylborohydride (KS-

selectride) und lithium-triamylborohydride (LS-selectride), most preferably by sodium cyano boro hydride.

It is especially preferred that the imine is reduced by $NaBH_3CN$ at a pH values of 6 – 7.

It is also preferred that in step b) or step a') the one or more activated carboxylic group(s) of an oligosaccharide S are activated carboxylic group(s) selected from the group consisting of a lactone, an anhydride, a mixed anhydride, and a halogenide of a carboxylic acid.

Suitable linker molecules are those that have at one end any reactive functional group that reacts with the component X and at the other end any reactive functional group that is able to react with an oligosaccharide S. Preferably, said bifunctional linker reacts with an amine, alcohol, thiol, aldehyde, carboxylic acid, or activated carboxylic acid of X and S.

Many suitable linkers are known in the art. Preferably, the suitable linkers form an amide, imine, secondary amine, ester, thioester, urea, carbonate, and/or carbamate bond

In a preferred embodiment, the bifunctional linker is preferably non-toxic and physiologically acceptable. More preferably, the bifunctional linker comprises a linear or branched aliphatic chain, preferably an aliphatic chain of 1 to 20, more preferably 1 to 12, most preferably 2 to 6 carbon atoms.

Particularly preferred bifunctional linkers are selected from the group consisting of:

a) linker molecules that connect an –SH group with an amino group, preferably derived from a compound selected from the group consisting of:

AMAS	(N-α(Maleimidoacetoxy)succinimide ester),
BMPS	(N-ß(Maleimidopropyloxy)succinimide ester),
GMBS	(N-γ(Maleimidobutyryloxy)succinimide ester),
EMCS	(N-ε(Maleimidocaproyloxy)succinimide ester),
MBS	(m-Maleimidobenzoyl-N-hydroxysuccinimide ester),
SMCC	(Succinimidyl4-(N-maleimidomethyl)-cyclohexane-1-carboxylate),

SMPB	(Succinimidyl-4-(p-maleimidophenyl) butyrate),
SPDP	(Succinimidyl-3-(2-pyridyldithio) propionate),
Sulfo-GMBS	(N-(γ-Maleimidobutyryloxy)sulfosuccinimide ester), and
Sulfo-EMCS	(N-(ε-Maleimidocaproyloxy)sulfosuccinimide ester);

b) linker molecules that connect two –SH groups, preferably derived from a compound selected from the group consisting of:

BMB	(1.4-Bis-maleimidobutane),
BMDB	(1.4-Bis-maleimido-2.3-dihydroxybutane),
BMH	(Bis-maleimidohexane),
BMOE	(Bis-maleimidoethane),
DTME	(Dithio-bis-maleimidoethane),
HBVS	(1.6-Hexane-bis-vinylsulfone),
$BM(PEO)_3$	(1.8-Bis-maleimidotriethyleneglycol), and
$BM(PEO)_4$	(1.11-Bis-maleimidotetraethyleneglycol);

c) linker molecules that connect two amino groups, preferably derived from a compound selected from the group consisting of:

BSOCOES	(Bis-(2-(succinimidyloxycarbonyloxy)-ethyl)sulfone,
BS^3	(Bis-(sulfosuccinimidyl)suberateDFDNB (1.5-Difluoro-2.4-dinitrobenzene),
DMA	(Dimethyladipimidate 2 HCl),
DSG	(Disuccinimidyl glutarate),
DSS	(Disuccinimidyl suberate), and
EGS	(Ethylene glycol bis(succinimidylsuccinate),

d) linker molecules that connect an –SH group and a –CHO functional groug, preferably derived from a compound selected from the group consisting of:

BMPH	(N-(ß-Maleimidopropionic acid)hydrazide TFA),
EMCH	(N-(ε-Maleimidocaproic acid)hydrazide),

KMUH (N-(κ-Maleimidoundecanoic acid)hydrazide),
M_2C_2H (4-(N-Maleimidomethyl)cyclohexane-1-carboxylhydrazide HCl),
MPBH (4-(4-N-Maleimidophenyl)butyric acid hydrazide HCl), and
PDPH (3-(2-Pyridyldithio)propionyl hydrazide),

e) linker molecules that connect an –SH group to an –OH group, preferably a compound derived from PMPI (N-(p-Maleimidophenyl)isocyanate);

f) linker molecules that connect an –SH group to a –COOH group, preferably derived from a compound selected from the group consisting of:
BMPA (N-ß-Maleimidopropionic acid),
EMCA (N-ε-Maleimidocaproic acid), and
KMUA (N-κ-Maleimidoundecanoic acid);

g) linker molecules that transform an amino group into a carboxyl group, preferably derived from a compound selected from the group consisting of: MSA (Methyl-N-succinimidyladipate) and its longer and shorter chain homologues or the corresponding ethylene glycol derivatives;

h) linker molecules that transform a –COOH group into an amino group, preferably derived from a compound selected from the group consisting of: DAB (1.4-Diaminobutane) or its longer and shorter chain homologues or the corresponding ethylene glycol derivatives.

The direct (by amide, ester, imine, secondary amine, carbonate, carbamate, urea, or thioester bond) or indirect (by bifunctional linker molecules) coupling products can be analyzed by standard chromatographic methods (such as, e.g., HPLC, TLC) and be fully characterized using MS, IR or NMR. This is a substantial advantage versus polymer conjugation. The reaction product of the oligosaccharides is clearly defined because it is a unique entity and not the sum of many polydisperse homologues as they are in the field of polymer conjugation. Thereafter, purification, isolation and characterisation techniques become more effective when working with the oligosaccharide conjugates of the present invention.

Component X is that part of the conjugates according to the invention that mediates its pharmaceutical utility. Therefore, conjugates of the present invention will be pharmaceutically active, too, and in addition provide the advantages over the pharmaceutically active component alone that have been described in more detail above.

Thus, in a further aspect the present invention relates to compound according to the invention for use as a medicament.

In addition, the present invention relates to a pharmaceutical composition comprising at least one of the compounds according to the invention and a pharmaceutically acceptable carrier.

It is particularly preferred to formulate the compounds of the invention by freeze-drying. On one side, freeze drying is a preferred dehydration and purification step. On the other side, freeze-drying will enhance the stability of saccharide compositions.

Therefore, the present invention relates to freeze-dried pharmaceutical composition comprising at least one of the compounds according to the invention and a pharmaceutically acceptable carrier.

The dehydrated, in particular freeze-dried pharmaceutical compositions may be regenerated to be ready for use by adding at least one physiologically acceptable aqueous solvent, such as water, physiological saline or any other suitable aqueous formulation.

In this respect, the present invention also relates to a kit comprising at least one of the compounds according to the invention in a dehydrated form, preferably in lyophilized form, and at least one physiologically acceptable aqueous solvent.

In pharmaceutical compositions the molar ratio of oligosaccharide and pharmaceutically active substance is preferably in the range from 20:1 to 1:1, preferably from 5:1 to 1:1.

In general, the compounds of the present invention demonstrate high solubility and stability in aqueous solutions and also in physiological media *in vitro*. Depending on the therapeutical need the compounds of the present invention can in be designed to be

cleaved very rapidly in plasma and esterase solutions, a few of them even quantitatively within a few minutes (e.g. in case of ester bonds) or act as slow release forms of the drug (e.g. when bonded as amide), thus providing excellent prodrugs (i.e. conjugated drugs that display their pharmaceutical effect only after being released in the free form). The compounds of the present invention are also efficacious *in vivo*. Some acting as prodrugs are readily hydrolyzed in the bloodstream. Others will be hydrolyzed very slowly, thus providing slow release forms of the pharmaceutically active compound. Alternatively even others will retain their pharmaceutical activity while conjugated.

In effecting treatment of a mammal in need of pharmaceutical action, the compounds disclosed by the present invention for said purpose can be administered in any form or mode which makes the therapeutic compound bioavailable in an effective amount, including oral or parenteral routes. For example, products of the present invention can be administered by enteral (oral, sublingual, buccal and rectal), parenteral (intradermal, subcutaneous, intramuscular, intraperitoneal, intravenous, intraarterial, intraarticular, intrathecal and epidural), topical (creams, ointments, lotions, transdermal patches, eye drops, inhalants, vaginal creams, rings and sponges, implants), intranasal, buccal, rectal routes, and the like.

Parenteral administration of the compounds of the present invention is preferred.

One skilled in the art of preparing formulations can readily select the proper form and mode of administration depending upon the particular characteristics of the product selected, the disease or condition to be treated, the stage of the disease or condition, and other relevant circumstances. (Remington's Pharmaceutical Sciences, Mack Publishing Co. (1990)). The products of the present invention can be administered alone or in the form of a pharmaceutical preparation in combination with pharmaceutically acceptable carriers or excipients, the proportion and nature of which are determined by the solubility and chemical properties of the product selected, the chosen route of administration, and standard pharmaceutical practice. Non-limiting examples of acceptable carriers or excipients are, e.g. binders, coatings, fillers, compression and encapsulation aids, disintegrants, creams and lotions, lubricants, materials for chewable tablets, parenterals, plasticizers, powder lubricants, soft gelatin capsules, spheres for coating, spheronization agents, suspending and gelling agents, sweeteners, wet granulation agents. For oral application suitable preparations are in the form of tablets, pills, capsules, powders, lozenges, sachets, cachets, suspensions, emulsions, solutions,

drops, juices, syrups, while for parenteral, topical and inhalative application suitable forms are solutions, suspensions, easily reconstitutable dry preparations as well as sprays. Compounds according to the invention in a sustained-release substance, in dissolved form or in a plaster, optionally with the addition of agents promoting penetration of the skin, are suitable percutaneous application preparations. The products of the present invention, while effective themselves, may be formulated and administered in the form of their pharmaceutically acceptable salts, such as acid addition salts or base addition salts, for purposes of stability, modulation of hydrophobicity, increased solubility, and the like.

The amount of active agent to be administered to the patient depends on the molecular weight and toxicity of the drug, the patient's weight, on the type of application, symptoms and the severity of the illness. Normally, 0.1 mg/kg to 25 mg/kg of at least one substance of the present invention is administered but when applied locally, e.g. intracoronary administration, much lower total doses are also possible.

Figures

Fig. 1 shows the results of the inhibition test with modified amphotericin B according to example 9. Assay conditions according to DIN 58940. Mlt-AmpB=maltotrionic acid conjugated amphotericin B. Clear wells indicate no growth of the test organism *Candida albicans* (i.e. positive effect of AmpB). Turbid wells indicate growth of the test organism (i.e. no effect of AmpB). MIC = minimal inhibitory concentration

The following examples further illustrate the best mode contemplated by the inventors for carrying out their invention. The examples relate to preferred embodiments and are not to be construed to be limiting on the scope of the invention.

EXAMPLE 1: Selective oxidation of maltotriose reducing end

In a round bottom flask one gram of maltotriose (~2 mmol) was dissolved in distilled water (1.0 ml). Thereafter 2.0 ml of a 0.1 N I_2 solution were added and the solution became brown. A 2 ml pipette containing 2.0 ml 1 N NaOH solution was then connected to the flask using a two ways connector, and the NaOH solution was dropped in, once

every four minutes (each drop having the volume of ~20 µl). After adding almost 0.2 ml of the NaOH the solution started to become clear again, then the second 2 ml portion of 0.1 N I_2 solution had to be added. At the end of this process 50 ml a 0.1 N I_2 solution and 7.5 ml of 1 N NaOH solution was used.

The reaction was then stopped, acidified with 2.0 N HCl solution, and extracted several times with ethyl ether in order to remove any I_2 left. At the end the solution was passed directly through the cation exchanger IR-120 H^+, and then incubated overnight in presence of silver carbonate in order to eliminate any excess of iodine/iodide. Thereafter the filtrate was passed once more through the same cation exchanger before being lyophilised. The final yield was found to be 85% and 95%.

EXAMPLE 2: Coupling of Amphotericin B to maltotrionic acid

46.61 mg of maltotrionic acid lactone and 21.31 mg of Amphotericin B (ratio ~ 4:1) were dissolved in 1.0 ml of anhydrous DMSO under inert atmosphere (argon). The temperature was increased up to 70°C and the reaction was allowed to run protected from light under moderate stirring conditions for 24 h. Thereafter the reaction was stopped by adding 20 ml of cold acetone which precipitates the coupling product. The precipitate was then washed once more with cold acetone, then with methanol and finally again with acetone, before being dissolved in water and lyophilised. The coupled product has a drug content (estimated by UV absorption at 410 nm) of 120 µg per mg.

Analogous reactions have been performed also with Mepartricin and with Nystatin.

EXAMPLE 3: Coupling of Neomycin to maltotrionic acid

In a two-necked round bottom flask, 64 mg of Neomycin and 52 mg of maltotrionic acid lacton were dissolved in 2.0 ml DMSO. After increasing the temperature up to 70°C, the reaction ran for 24 h under inert atmosphere (argon). The reaction was finally stopped and the coupling product precipitated by adding cold acetone. The precipitate was washed one more time with methanol and finally again with acetone. After dissolving it in water the product was lyophilized to yield 108 mg of coupling product (yield 93%).

EXAMPLE 4: Coupling of Daunorubicin to maltotrionic acid

In a two-necked round bottom flask 0.7 mg of Daunorubicin were dissolved in 1 ml DMSO together with 62.41 mg of maltotrionic acid lacton and 0.152 mg of DMAP. The reaction ran at 70 °C, for 24 h under argon atmosphere and moderate stirring. The reaction was stopped by adding cold acetone which precipitates the conjugate. After centrifugation the solid pellet is resuspended in acetone several times until the filtrate did not show any more red coloration. The pellet is finally dissolved in water and lyophilised. The purity of the coupling product was checked by RP–HPLC and the drug content was determined by UV photometry. The coupling product contains 0,4 µg Daunorubicin per mg. The yield was 78%.

EXAMPLE 5: Coupling of Propofol to lactobionic acid

a) Synthesis of succinic acid mono-propofol ester

In a 50ml round bottom flask 1 ml of propofol has been stirred with 2.5ml of TEA at room temperature. When the mixture looked homogeneous 5.5 mmol of succinnic anhydride were added. The reaction was allowed to proceed under moderate stirring conditions for 22 h. The progress of the reaction was followed by TLC monitoring or by simply observing the disappearance of succinic anhydride whose solubility in the mixture is low, so most of it remained in the reaction vessel as a white solid. After 22 h the reaction was stopped, the solution looked brownish. After elimination of most of the TEA under vacuum, 10 ml of 0.2 N HCl were added to the solution which was vigorously stirred and kept in an ice bath for 30 min. Thereafter the white swaying precipitate was removed from the reaction by filtration through a proper funnel filter. The precipitate was dissolved once more in EtOH and precipitated a second time by adding cold water, filtrated and kept at –20 °C.

b) Synthesis of lactobionic acid hydrazide

Three grams of lactobionic acid were dissolved in 5 ml of warm DMSO (~70 °C). After the complete dissolution 7.5 mmol of mono chloride salt of hydrazine were added to the reaction vessel. The solution was stirred at 45 °C for 20 h. The proceeding of the hydrazide formation was monitored using TLC coupled with a ninhydrin test to reveal the

presence of free amino groups. The protonated amine appeared yellow in the ninhydrin test. When the reaction seemed complete, it was stopped by adding an excess of water and then 0.1 N NaOH solution was inserted dropwise until a pH~10 was reached in order to neutralise the HCl. The mixture was frozen and lyophilised. The dry product was then dissolved in water and lyophilised once more to eliminate the last traces of DMSO.

c) Alternative synthesis of lactobionic acid hydrazide

Three grams of lactobionic acid lactone were dissolved in 5.0 ml of warm DMSO (~70°C). Once dissolved 1.0 gram of monoBOC–Hydrazine was added to the reaction vessel. The reaction ran for 16h under inert atmosphere (argon) and was monitored by TLC (eluent CH_3Cl). When the spot of the BOC–hydrazine disappeared the reaction was stopped, cooled down to 4-5°C and extracted with water – chloroform several times. The aqueous phase was finally degassed and lyophilized. The product dissolved in MeOH has been deprotected from the BOC- function by bubbling HCl gas into the solution for 30'. The deprotection was also monitored by TLC. At the end the solvent was completely evaporated, the solid was washed three times with ethyl ether in order to remove completely the remaining HCl and finally dissolved in water and lyophylized. The hydrochloride salt was characterised by ESI-MS.

d) Synthesis of lactobionic acid diamino butanamide

Three grams of lactobionic acid lacton were dissolved in 3.0 ml of warm DMSO (~70°C). In a separate vessel a 30 times molar excess of diamino butane was dissolved in 2.0 ml of DMSO and then added to the first solution. The reaction was left under argon overnight under moderate stirring. The monitoring of the reaction was done by TLC. After stopping the reaction by adding 30 ml of NaOH sol. 0.01 N, this solution was extracted with a mixture chloroform / ethyl acetate 4:1 several times. The organic phase, washed two times with water was eliminated, while the aqueous phase, after degassing, was lyophilized. The product showed the calculated mol peak in ESI-MS.

e) Final coupling

One mmol of the succinic acid mono-propofol ester and one mmol of the lactobionic acid amino derivative (from reaction b, c, or d) were dissolved in 3ml of DMF and stirred at room temperature. The temperature was decreased to 0°C and a 1:1 molar amount of

DCC was added to the chilled solution. The reaction was allowed to run one hour under these conditions before increasing gradually the temperature to 25°C. The reaction was monitored by TLC coupled with a ninhydrin test. The disappearance of the free amino functions indicated the end of the reaction (normally after 2 h). The reaction was then stopped by adding dilute HCl. The precipitate was washed three times with cold water and then eliminated. The aqueous fractions were frozen and lyophilized. The purity of the product was checked by TLC, confirmed by RP–HPLC (C_{18}), and its characterisation was done by ESI-MS.

EXAMPLE 6: Coupling of Propofol to glucosamine

In a two necked round bottom flask 1.8 mmol of succinic acid mono-propofol ester is dissolved in 2.0 ml of MeOH. The solution is then chilled in an ice bath. A 5 times molar excess of CDI is then added to the solution and allowed to run in the same conditions for 15 min. With the help of a dropping funnel an equimolar solution of glucosamine in 2 ml of MeOH was slowly added during 10 min. Thereafter the reaction was allowed to proceed for one more hour on ice and then overnight at room temperature. The reaction is monitored by TLC. The reaction was finally stopped by adding 10 ml of a cold 0.1 N HCl solution, filtered and passed through a cation exchanger column filled with IR-120 H^+. The eluate is finally lyophilised and the purity is checked by RP–HPLC. The product was characterized by ESI-MS and NMR.

EXAMPLE 7: Coupling of Propofol to maltotrionic acid

In 2.0 ml of a 3:1 DMSO : MeOH mixture were dissolved 200 mg of Propofol, a three times molar excess of maltotrionic acid, and a catalytic amount of TEA. The solution was left stirring at room temperature for 10 min. In a separate vessel 350 mg of DCC were dissolved in 1 ml of the same solution and added dropwise to the previous mixture during a 3 min. time period. The reaction was warmed up to 60°C and allowed to run under these conditions for 20h. Finally it was stopped and then filtrated. The coupling product was recovered by precipitation in acetone (50 ml) and washed several times with EtOH (100 ml), ethyl acetate (100 ml) and finally acetone (100 ml). The reaction has been monitored by TLC and the purity of the product has been confirmed also by RP–HPLC on a C–18 column.

EXAMPLE 8: Coupling of Propofol to glucuronic acid

In a two necked 50 ml round bottom flask 10 mmol of glucuronic acid were dissolved in 2.0 ml of DMF. An equimolar amount of TEA was added and the solution was cooled down in an ice bath. Then 12 mmol of isobutyl chlorocarbonate were added and the reaction was kept cold for 30 min. In a separate vessel 10 mmol of propofol were mixed with 0.5 ml of TEA and then added dropwise to the first solution with the help of a dropping funnel. The reaction run for 1 day at 4°C and overnight at room temperature. It was monitored by TLC. After stopping the run the solution was evaporated yielding a brown oily product which was dissolved in water and extracted several times with chloroform. The organic phase, washed two times with water can be eliminated. The aqueous phase, after degassing, was passed through a mixed ion exchanger before being lyophilised. The purity was checked by RP–HPLC and the product has been characterised by ESI-MS and NMR.

EXAMPLE 9: Coupling of Propofol to gluconic acid

a) Synthesis of gluconic acid hydrazide

Five grams of gluconic acid were suspended in 15 ml of warm MeOH. After the complete dissolution 4 grams of monoBOC-hydrazine were added to the reaction vessel. The solution was refluxed for 36 h. After the addition of monoBOC-hydrazine the suspension disappeared. At the end, the reaction showed a white precipitate which contained most of the product. The solvent was removed under vacuum and the solid product was extracted in a mixture of NaOH sol. 0.1 N / chloroform. The organic phase was removed while the aqueous phase was passed through anionic exchanger resin and lyophilized. Finally, product was dissolved in cold MeOH and deprotected from the BOC- function by bubbling HCl gas into the solution over 30'. The purity of the protected and the deprotected product was checked by TLC, HPLC and the identity was confirmed by ESI-MS.

b) Final coupling

1 mmol of the succinic acid mono-propofol ester (from reaction a) of example 5) and 1 mmol of the gluconic acid amino derivative (from reaction b) were dissolved in 3 ml of DMF and stirred at room temperature. Then, a 1.5 molar excess of CDI (1,1'-Carbonyldiimidazole) was added to the reaction vessel together with 150 µl of TEA. The reaction was allowed to proceed overnight under these conditions. The reaction was monitored by TLC coupled with a ninhydrin test. The disappearance of the free amino functions indicated the end of the reaction. The reaction was then stopped and the solvent evaporated under vacuum. The residue, still oily, was purified by flash chromatography on silica gel using a mixture $CHCl_3$/MeOH as eluent. The purity of the final product was checked by TLC, confirmed by RP–HPLC (C_{18}), and its characterisation was done by ESI-MS.

EXAMPLE 10: Coupling of Propofol to modified glucose

a) Synthesis of an amino derivative of glucose

5 g glucose and 3.5 mg monoBOC-Hydrazine were dissolved in 60 ml of a 5:1 MeOH-water mixture in a 250 ml round bottom flask. The solution was briefly warmed to 60 °C (5 - 10 minutes, optional) and then was allowed to reach room temperature slowly. Thereafter, 2.6 g of $NaBH_3CN$ were added and the pH was corrected to 6 - 7 by adding a few drops of a 2N HCl solution. The reaction was allowed to run for 12 days while checking and correcting the pH to 6 - 7 by means of a 2N HCl solution daily. The reaction was considered finished when no shift in the pH value was observed. At the end, the MeOH was evaporated under vacuum and the water solution was extracted with $CHCl_3$ several times. The aqueous phase was then degassed, incubated in the presence of anionic exchanger and lyophilized. Finally, the solid product was dissolved in a 8:2 $CHCl_3$/MeOH mixture and extracted on silica gel. The organic solvents were evaporated, the product was dissolved in water and again lyophilized. 6.56 g purified product, corresponding to a yield of 79% were obtained. The product was characterised by ESI-MS. Part of this product was deprotected from the BOC-function and another part was utilised as such, postponing the deprotection after the final coupling step. In both cases the deprotection was carried out by dissolving the product in cold MeOH and bubbling HCl into this solution for 30'.

b) Final coupling

Three mmol of the succinic acid mono-propofol ester (from step a) of Example 5) and three mmol of the 1-deoxy-1-hydrazinoglucitol (from reaction b) were dissolved in 10 ml of DMF and stirred at room temperature. Then, a 1.5 molar excess of CDI was added to the reaction vessel together with 150 µl of TEA. The reaction was allowed to run overnight under these conditions. The reaction was monitored by TLC coupled with a ninhydrin test. The disappearance of the free amino functions indicated the end of the reaction. The reaction was then stopped and the solvent was evaporated under vacuum. The residue, still oily, was purified by flash chromatography using a mixture $CHCl_3$/MeOH as eluent on silica gel. The purity of the final product was checked by TLC, confirmed by RP–HPLC (C_{18}), and its characterisation was done by ESI-MS.

Also, the same reaction was performed with the BOC-protected 1-deoxy-1-hydrazinoglucitol.

EXAMPLE 11: Determination of solubility and antimycotic activity of maltotrionic acid coupled Amphotericin B (Mlt–AmpB)

In order to show the effectiveness of Amphotericin B after covalent coupling to maltotrionic acid the conjugate was checked by its growth inhibition potential of the pathogen Candida albicans according to a standardised procedure (DIN 58940).

Test Method

Bouillon dilution method carried out in 96 well microtitration plates according to E DIN 58940 Medical microbiology – Susceptibility testing of pathogens to antimicrobial agents, Part 84: Microdilution – Special requirements for testing of fungi against antifungal agents. Results are given as MIC (minimal inhibitory concentration). The MIC is the lowest concentration where no visible fungal growth can be detected in the tested sample.

Test Conditions

Strain: *Candida albicans* DSM 11943
Inoculum: *C. albicans* 5 * 10^4 KBE/ml in High Resolution Medium (Oxoid)
Incubation Temperature: 30°C
Sample volume: 100µl
Test volume: 200µl (100µl sample + 100µl inoculum)

Tested Substance/ MIC desired value

Tested Substance:
Mlt-AmpB, drug content: 2,05µg per mg.
Amphotericin B from Alpharma, Denmark, Lot 1970005.

After dissolving the freeze-dried coupled product in PBS (Phosphate Buffered Saline) the solution was filtered through a sterile non-pyrogenic filter (Whatman, 13mm syringe filter, polysulfone, 0.2µm) and subsequently a MIC test was carried out. The MIC desired value according to DIN 58940 should be between 0.125 and 1.0 µg/ml.

The result (see Fig. 1) shows that the coupled Amphotericin B has partially lost the original activity, nevertheless it still stays in the accepted activity range. It should be to recognized that the conjugate is completely soluble, since no difference can be observed among the sterile filtered and the non filtered material.

Therefore the loss in potency can be easily compensate with the big increase in solubility. In fact, the conjugate presents a solubility in water higher than 800 mg per ml, which results in an amount of drug in solution of almost 100 mg in 1ml while the water solubility of the Amphotericin as such is around 0.1 mg per ml and only using solution with extreme pH values. In comparison Mlt-AmpB has a 1000 fold improved water solubility.